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Variation in resource consumption across a gradient of increasing intra- and interspecific richness

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Abstract. Based on the premise that ecosystems with more species will function at more efficient rates, declining biodiversity is expected to alter important ecosystem functions, goods, and services across the globe. However, applicability of this general hypothesis to genetic or clonal richness in assemblages composed of few species is understudied. This illustrates the need to expand the focus of biodiversity–ecosystem-function experiments across all levels of biological diversity (including genetic). To explore this generality, we manipulated intraspecific (clonal) and interspecific (species) richness of a primary consumer, *Daphnia*, and measured assemblage feeding rate and total resource consumption. Our results showed that greater clonal richness had no effect on *Daphnia* feeding, and greater species richness decreased feeding-related effects of *Daphnia*. This suggests that multiclonal *Daphnia* assemblages may be no more efficient at consuming resources than monocultures, and that monocultures of *Daphnia* may consume resources more efficiently than more species-rich assemblages. The inhibitory effect of increasing richness observed in this study resulted from chemical and mechanical interference among some of the *Daphnia* taxa. This suggests that consumer-mediated ecosystem functions could be reduced when assemblages include taxa equipped with adaptations for interference competition.

Key words: allelopathy; biodiversity ecosystem function; complementarity; Daphnia; *feeding rate; freshwater lake; functional richness; genetic richness; microcosm experiment; selection; species richness.*

Introduction

The number of species on Earth is declining rapidly and may even exceed rates of previous mass extinctions (Millennium Assessment 2005). The ecosystem-level consequences of this modern extinction are uncertain. However, recent theoretical and empirical advances have provided the framework for a contemporary hypothesis linking species richness and ecosystem functioning (Loreau et al. 2001, Naeem 2002). The general prediction stemming from this biodiversity–ecosystem-function hypothesis (hereafter BEF) is that ecosystems with more species are likely to use resources more completely and therefore have greater rates of biomass accumulation (Fox 2005). This has been supported experimentally in plant, herbivore, detritivore, and predator communities from both aquatic and terrestrial ecosystems (Balvanera et al. 2006, Cardinale et al. 2006, Duffy et al. 2007).

Two general mechanisms explain the positive effect of increasing diversity on ecosystem functioning: selection effects and complementarity effects (Loreau and Hector 2001). Selection effects include any diversity-related effect driven solely by the production of an individual species. This can occur when a single, highly productive species dominates in more diverse assemblages and limits production of other taxa via competition or other interactions. Thus, diversity can drive ecosystem functioning by this mechanism if the probability of including dominant taxa in an assemblage also increases with diversity (Aarsen 1997, Huston 1997). Alternatively, complementarity effects represent diversity-related changes in ecosystem functioning that cannot be explained by individual taxa. Because complementarity includes the net balance of all biological interactions, such as habitat partitioning and non-additive species interactions, ecosystem functioning in more diverse assemblages should increase with greater frequency of multispecies interactions (Petchey 2003). Recent syntheses of the existing literature have demonstrated that both selection and complementarity are simultaneously driving the diversity-related effects on ecosystem functioning across systems (Cardinale et al. 2006, 2007). Therefore, based on growing support for these predictions, a modern extinction event may affect biomass production or consumption of limiting resources across ecosystems by eliminating functionally important taxa from communities and reducing multispecies interactions.

Although most BEF studies have focused on ecosystem effects of increasing functional group or species

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richness, many ecosystems have communities with only one or a few dominant taxa. These ecosystems often have large spatial extents and their functioning provides goods and services at landscape scales. For example, cattail marshes, sea grass beds, and kelp forests are dominated by only a few plant species, but the ecosystem-level functions of these communities provide an array of ecosystem services (Hughes and Stachowicz 2004, Reusch et al. 2005, Crutsinger et al. 2006, 2008). Because of the importance of these systems and pervasive threats to these communities, a number of recent experiments have tested whether richness at finer scales (i.e., genetic or clonal richness) may influence rates of ecosystem functioning through mechanisms analogous to traditional BEF experiments. These studies suggest that greater genetic richness can drive several ecosystem functions such as productivity, response to disturbance, invasibility, secondary production, decomposition, and nutrient dynamics (reviewed by Hughes et al. 2008). The majority of these experiments have focused on plant communities because of their potential for large clonal populations with varying degrees of genetic richness. It is clear there is need to expand this effort to consumers that also form large, clonal populations and regulate ecosystem functioning through their foraging activity.

Daphnia are ubiquitous primary consumers in freshwater and marine ecosystems. They forage on phytoplankton and bacteria at high rates, are the principal resource for many predators, and, thus, provide a direct link between primary and secondary production (Persson et al. 2007). However, *Daphnia* are trophically and functionally similar (Kerfoot et al. 1985) with assemblages that often have low species richness (Shurin et al. 2007) or are dominated by large, single-species populations of clonal individuals (Weider et al. 1999). Thus, richness-related effects on *Daphnia* feeding are likely to be linked to either fine-scale differences among species or among genetically unique clones. For example, variation in the spacing of setae on the filter-feeding appendages may reduce resource overlap in *Daphnia* assemblages, making it plausible to predict that increased species richness in an assemblage will positively affect total assemblage feeding rate (Norberg 2000). Further, clonal variation within a *Daphnia* species also may regulate rates of feeding in a manner similar to genetic regulation of resource use and productivity documented in plant assemblages (Crutsinger et al. 2006). Because feeding rates of *Daphnia* assemblages can directly regulate the flux of energy from basal trophic levels to higher order consumers, understanding whether there are effects of species or clonal richness on feeding rates in these organisms could provide further evidence that biodiversity at the finest scale (i.e., genetic) can drive ecosystem functioning across biota and ecosystems.

Herein, we report the effects of increase in genetic richness on *Daphnia* feeding. We show that increasing clonal richness in *Daphnia* had no effect on feeding rate or total resource depletion, but that increasing species richness reduced *Daphnia* feeding via a combination of chemical and mechanical inhibitory mechanisms. These results differed from our predictions, which specifically stated (1) that feeding rates would be lowest in monoculture assemblages composed of individual clones, that feeding rates would (2) increase in assemblages composed either of different clones from within the same population or (3) increase in assemblages composed of clones from different populations, relative to monoculture assemblages, and (4) that assemblages with multiple *Daphnia* species would have the greatest feeding rates.

Methods

Experimental organisms and richness treatments

We used clonal lineages of four *Daphnia* species (see Plate 1) to test the effect of increasing genetic richness on feeding in *Daphnia* assemblages (Appendix A). The experimental populations for each clone were from long-established lineages kept in the laboratory at the University of Oklahoma Biological Station (UOBS; Marshal County, Oklahoma, USA), and maintained as separate populations in 20-L plastic buckets. *Daphnia* were grown in COMBO, a nutrient-rich medium developed for simultaneous culture of both algae and zooplankton (Kilham et al. 1998), and fed the green alga *Scenedesmus acutus* every two days. *Scenedesmus acutus* was cultured in chemostats using COMBO with the addition of trace elements (Kilham et al. 1998).

Daphnia are cyclical parthenogens (alternating between sexual and asexual life cycles). Individuals can produce a clutch of clonal offspring about every three to four days, which mature in about 5 to 10 days. Therefore, a population of genetically identical daphniids (assuming no mutations) can originate from a single mother and reach high density in a relatively short time. *Daphnia* are suspension feeders, filtering particulates from the water using specialized appendages and filtering setae. The amount of water cleared of particulates through filtering activity (clearance rate) changes proportionally with daphniid density and biomass. Therefore, mass-specific feeding rates for *Daphnia* assemblages can be easily determined by establishing a density gradient and measuring resulting change in particle concentration from the water column (Lehman 1980). These aspects of *Daphnia* biology make this system ideally suited for testing short-term (24 h), intra- and interspecific richness effects on feeding rates in a laboratory setting.

We measured feeding rates of different *Daphnia* assemblages that represented four increasing levels of genetic richness. The first level included *Daphnia* assemblages composed of all individuals that originated from the same mother (i.e., clone) and, thus, had zero genetic richness, hereafter referred to as monocultures. The second level of genetic richness included assemblag-

es composed of all possible combinations of four *D. pulex* clones. These four clones originated from separate mothers that were isolated from the same source population, and hereafter, this level of richness is referred to as intrapopulation clonal richness. The third level of genetic richness included assemblages composed of all possible combinations of four *D. pulicaria* clones. These four clones originated from different mothers from four different source populations, and hereafter, this level of richness is referred to as interpopulation clonal richness. Finally, the fourth level of genetic richness included single clones from four different *Daphnia* species. We refer to this level of richness as interspecific richness. We manipulated richness within each of these levels (except the monocultures) by increasing the clonal or species richness from two, three, or four clones or species per assemblage (Appendix B). We measured the feeding rate of all *Daphnia* in monoculture, and in all possible two-, three- and four-clone or species (hereafter called "taxa") combinations within each level of genetic richness (i.e., intrapopulation, interpopulation, and interspecific).

Experimental setup

To measure feeding rates by *Daphnia*, we used density-gradient bottle experiments, in which the volume of water cleared of food particles (here *S. acutus*) by feeding *Daphnia* (clearance rate) was measured as a proxy for mass-specific feeding rate. We also estimated total chlorophyll consumed (TCC) by each *Daphnia* assemblage by calculating the gross change in chlorophyll in the experimental units over time. Experiments were conducted in clear, plastic 2.5-L Nalgene bottles filled with 2 L of nutrient-rich COMBO, allowing for an unbiased estimate of *Daphnia* clearance rates (CR) and TCC without the confounding positive effects of *Daphnia*-mediated nutrient mineralization. To begin each experiment, we first removed a subsample of *Daphnia* from the stock buckets using a 200-μm plankton net and placed the individuals into a sorting tray with COMBO. We randomly assigned density treatments to each of the bottles (three replicates per density treatment), selected adult daphniids of the same size and without developing broods from the tray, and added the necessary number of individuals to each bottle using a wide-bore pipette. After daphniids were added to the bottles, we added ~5–10 mL of concentrated *S. acutus* to each bottle to achieve an initial in vivo chlorophyll concentration of ~6–7 μg/L. Bottles were inverted several times to mix the *S. acutus* with the media, and 5-mL water samples from each bottle were analyzed for initial chlorophyll (A_0) concentrations using a Turner model TD 700 bench top fluorometer (Turner Designs, Sunnyvale, California, USA). Bottles were moved to an environmental chamber and laid on their side on a roller system. The roller system rolled (at four revolutions per minute and alternating between clockwise and counterclockwise every minute) and tilted each bottle, creating a gentle turbulence that kept the *S. acutus* from settling during the course of each experiment. The bottles were rolled for 24 h under full-spectrum grow lights set at a 12 h light : 12 h dark cycle. Following each 24-h experiment, the bottles were removed from the roller system and three replicate water samples were taken from each bottle and analyzed for in vivo chlorophyll using fluorometry. Mean values from the three replicate water samples represented the chlorophyll in each bottle after 24 h (A_t). The *Daphnia* were filtered from the bottles with a 200-μm-mesh sieve and preserved in 2% formalin for length measurements. We measured total body length (excluding tail spines) of each daphniid recovered from bottles to the nearest 0.01 mm under a dissecting microscope and used a predetermined length–dry-mass regression per clone to estimate mass of each individual daphniid. The predetermined length–dry-mass regressions for each clone were calculated by measuring 20 individuals representing a range of sizes to the nearest 0.01 mm. We then put each daphniid individual in a small pre-weighed aluminum weighing pan. Pans plus daphniids were dried at 50°C for 24 h and reweighed to the nearest microgram using a Cahn model 27 electrobalance (ATI Cahn, Madison, Wisconsin, USA). We calculated total *Daphnia* biomass (Z) per bottle by summing individual masses for all daphniids recovered at the end of each experiment.

We calculated observed clearance rate (CR_o) based on the following regression:

$$r_{chl} = CR_o\,(Z) + B \tag{1}$$

where CR_o is the negative of the slope between change in chlorophyll ($r_{chl} = \ln[A_0 - A_t]/\Delta t$) over the course of the experiment ($t = 1$ day) and *Daphnia* biomass (Z, mg/L). Eq. 1 calculates biomass-specific CR_o while accounting for algae growth rate (B) over each 24-h experimental period (Lehman 1980).

To compare feeding among *Daphnia* assemblages independent of biomass, we also calculated observed total chlorophyll consumed TCC_o by each *Daphnia* assemblage. Because r_{chl} changed linearly with increasing *Daphnia* density, we calculated TCC_o for each assemblage using only the control and highest density treatment (100 individuals per experimental unit, Appendix B). We calculated TCC_o as the gross change in chlorophyll using the following equation:

$$TCC_o = (\Delta A_{Control}) - (\Delta A_{High}) \tag{2}$$

where $\Delta A_{Control}$ is the change in chlorophyll ($A_0 - A_t$) of control treatments (0 individuals), and ΔA_{High} is the change in chlorophyll ($A_0 - A_t$) of high density treatments (100 individuals).

Richness effects on Daphnia *feeding*

We conducted 10 experiments to estimate the CR_o of each clone in monoculture (Appendix A). In these experiments, we manipulated initial *Daphnia* density from 0, 6, 12, 24, and 50 individuals/L ($n = 3$ per density

treatment). Initial and final densities changed little during the 24-h experiments. Using r_{chl} and Z recovered per bottle, we calculated CR_o for each clone using Eq. 1. We compared feeding rates among the clones by contrasting CR_o using proc GLM (SAS Institute 2003). Using Eq. 2, we also calculated TCC_o for each of these clones in monoculture (Appendix C). We compared TCC_o among clones using ANOVA and a Tukey multiple comparison procedure (SAS Institute 2003).

We used the density-gradient approach to estimate the CR_o of each four-taxa assemblage at the intrapopulation, interpopulation, and interspecific richness level. In these experiments, we varied *Daphnia* density from 0, 6, 12, 24, and 50 individuals/L ($n = 3$ per density treatment) and kept the proportion of individual taxa equal across all densities (Appendix B). We calculated CR_o and TCC_o of each four-tax assemblage using Eqs. 1 and 2, respectively. We repeated each four-taxa CR experiment (i.e., two experiments) to verify results.

By keeping the proportion of taxa equal across a gradient of increasing *Daphnia* density (i.e., substitutive design; see Appendix B), we were able calculate the expected clearance rates (CR_e) for each four-taxa assemblage using a multiplicative risk model:

$$CR_e = \sum_{i=1}^{n}(Z_i \times CR_i) + \prod_{i=1}^{n}(Z_i \times CR_i) \qquad (3)$$

where CR_e for each assemblage is based on the proportional effects of all taxa in the assemblage (n) using the final biomass of each individual clone or species comprising the assemblage (Z_i), and the average monoculture CR_o calculated from Eq. 1 for each clone or species comprising the assemblage (CR_i). The upper and lower 95% CI for CR_e were calculated by substituting mean CR_i with the corresponding upper and lower 95% CR_o from Eq. 1. We calculated CR_e for each four-taxa assemblage using Eq. 3 and compared the CR_o of the four-taxa assemblages to the CR_e using a parameter coefficient test in Proc REG (SAS Institute 2003).

We also calculated expected total chlorophyll consumed (TCC_e) for each four-taxa assemblage also using a multiplicative risk model:

$$TCC_e = \sum_{i=1}^{n}\left(\frac{1}{n}\right)(TCC_i) - \prod_{i=1}^{n}\left(\frac{1}{n}\right)(TCC_i) \qquad (4)$$

where TCC_e for each assemblage is based on the proportional effects of all taxa in the assemblage (n), and the average monoculture TCC_o calculated from Eq. 2 for each clone/species comprising the assemblage (TCC_i). The upper and lower 95% CI for TCC_o were calculated by substituting mean TCC_i with the corresponding upper and lower 95% TCC_o from Eq. 2. We calculated TCC_e for each four-taxa assemblage using Eq. 4 and compared the TCC_o of the four taxa to the TCC_e using a one-sample t test (SAS Institute 2003).

A multiplicative risk model sums predatory effects of each individual in the assemblage and removes any unrealistic joint predatory effects that are inherently present in additive models (Sih et al. 1998). Although our experimental design likely did not violate assumptions of an additive model, i.e., resources were not depleted, we chose to use the multiplicative model because this model produced slightly more conservative estimates than the additive model.

We conducted a series of combination experiments, using all possible assemblage combinations of four *D. pulex* clones, four *D. pulicaria* clones, and four *Daphnia* species (one clone from each species), to estimate the effects of increasing intrapopulation clonal richness, interpopulation clonal richness, and interspecific richness on *Daphnia* assemblage CR and TCC (Appendix B). We were unable to use a density-gradient approach for these combination experiments because of the high number of experimental bottles needed to simultaneously test the various assemblage combinations and monocultures of taxa in each assemblage. Therefore, we used an endpoint approach (Moigis 2006) to estimate CR_o and TCC_o of all possible assemblage combinations and respective monocultures. This method included a no *Daphnia* treatment and a high density treatment (~50 individuals/L) as endpoints with three replicates per density treatment (Appendix B). We calculated CR_o by fitting a line between these two end-point densities using Eq. 1, and we calculated TCC_o using Eq. 2. Using Eqs. 3 and 4, we calculated CR_e and TCC_e, respectively, for each multi-taxa assemblage combination based on monoculture treatments that ran during the same trial as the respective combination treatments. We compared the CR_o and TCC_o for each combination to the corresponding CR*e* and TCC*e* using a parameter coefficient test statement in proc REG or one sample t test, respectively (SAS Institute 2003).

As opposed to plant systems, our ability to partition complementarity and selection was limited because we had no mechanism for determining individual species contributions in multispecies treatments (Loreau and Hector 2001, Cardinale et al. 2002). Therefore, we modified some published approaches to help evaluate potential complimentarity and selection effects. First, we calculated the difference between the CR_o and CR_e (ΔCR) and TCC_o and TCC_e (ΔTCC) and used this as an estimate of the net biodiversity effect of each multi-taxa assemblage on CR or TCC (Loreau and Hector 2001). Based on the null model that proportional effects of individual species will be identical in monoculture and multispecies treatments, we interpreted positive ΔCR or ΔTCC as evidence that mixtures consumed resources at a greater rate and more completely than the combined sum of all individual clones/species in the assemblage. We used a negative ΔCR or ΔTCC as evidence that mixtures consumed resources slower or less completely than the combined sum of each individual species. However, this effect could be driven by disproportional

effects of some taxa, i.e., the selection effect. To evaluate whether ΔCR or ΔTCC was possibly due to a selection effect, we compared ΔCR and ΔTCC for each assemblage combination to the best or worst performing clone/species in monoculture (Mikola et al. 2002). If the difference was zero, the net biodiversity effect could be influenced by selection of the best or worst performing clone. If this difference was larger or smaller than zero the net biodiversity effect could possibly be explained to some degree by facilitation or inhibition, respectively.

Chemical inhibition on clearance rates

To test for possible chemical inhibition of assemblage CR at the interspecific-level, we created conditioned water for each two-, three-, and four-taxa assemblage by adding the different *Daphnia* species to 20-L plastic buckets at a density of 24 individuals/L and allowing them to coexist for 48 h. After this time period, the conditioned water was filtered through Gelman A/E glass fiber filters (Pall Gelma, Ann Arbor, Michigan, USA) to be used for the conditioned water treatment in subsequent CR experiments, which included a conditioned and a non-conditioned (regular) water treatment. Using an endpoint approach, each *Daphnia* species from each assemblage combination was taken from separate stock populations and added to experimental bottles in monoculture (24 individuals/L), which contained either water conditioned by that respective assemblage or regular water. Each monoculture was replicated three times per water treatment. We calculated CR_o for each daphniid species in the conditioned and regular water treatments using Eq. 1, and compared CR_o between water treatments by analyzing the difference in CR_o using Proc GLM (SAS Institute 2003).

Results

There was no effect of increasing clonal richness on CR_o or TCC_o of *Daphnia* at the intrapopulation or interpopulation richness levels (Fig. 1A–D), and CR_o or TCC_o was not different from CR_e or TCC_e based on the multiplicative models from monocultures (Fig. 1A–D, Appendix D). At the interspecific richness level, CR_o and TCC_o decreased significantly with increased *Daphnia* richness, and most species combinations had significantly lower CR_o or TCC_o than CR_e or TCC_e predicted from monoculture assemblages (Fig. 1E and F; Appendix D). For example, the CR_o for two-, three-, and four-species assemblages was on average 40% ± 0.38% (mean ± SD), 49% ± 0.33%, and 79% ± 0.03% lower than the CR_e for each respective richness level. The TCC_o for two-, three-, and four-species assemblages was on average 41% ± 0.31%, 52% ± 0.26%, and 74% ± 0.06% lower than the TCC_e for each respective richness level. Thus, the net-richness effect (ΔCR and ΔTCC) of the multispecies assemblages increased with increasing *Daphnia* richness. The CR_o for three of the two-species combinations, two of the three-species combinations, and all of the four-species combinations were less than the poorest performing species in monoculture (Appendix D). The TCC_o for two of the two-species combinations, two of the three-species combinations, and all of the four-species combinations also were less than the poorest performing species in monoculture (Appendix D).

Chemical inhibition was a common cause for reduced CR_o in the interspecific richness experiments (Fig. 2). For example, most daphniids in the two-species combinations were inhibited to some degree by chemical constituents in the conditioned water, with the exception of *D. pulicaria* and *D. pulex*, which were only inhibited by *D. magna* (Fig. 2A). Similarly, many of the daphniids in three-species combinations showed reduced CR_o in conditioned water treatments. The exception was *D. pulicaria* which was only inhibited when *D. magna* and *D. obtusa* were both present (Fig. 2B). All daphniids had reduced CR_o in water conditioned by the four-species combination, suggesting that all species experience chemical inhibition at the greatest richness level. However, the decrease in CR_o in conditioned water experiments did not always account for the total decrease in CR_o in the combination experiments, suggesting that physical interference also likely caused reduced CR_o of the *Daphnia* assemblage (Appendix E). For example, in two-taxa combinations with *D. pulex–D. obtusa*, *D. pulicaria–D.obtusa*, and *D. pulex–D. magana* the reduction in feeding rate was greater than that from the conditioned water experiments, suggesting that both chemical and mechanical inhibition affected feeding in these taxa. Moreover, the four-species combination and two of the three-species combinations showed evidence that mechanical inhibition was occurring along with chemical inhibition (Appendix E).

Discussion

Our four predictions were not supported. Monocultures did not have the lowest feeding rates relative to other richness combinations (contrary to prediction 1). There was no effect of increasing clonal richness on feeding rates at the intrapopulation or interpopulation levels (contrary to predictions 2 and 3, respectively). *Daphnia* feeding rate was lowest in treatments with the greatest number of species (contrary to prediction 4).

The feeding rates measured in our monoculture and clonal treatments reflected feeding rates reported for *D. pulex*, *D. magna*, *D. obtusa*, and *D. pulicaria* in the literature (e.g., Burns 1969, Haney 1985, Kreutzer and Lampert 1999). Therefore, because feeding rates of multi-clonal assemblages at the intra- and interpopulation levels were similar to monocultures, our data suggest that increasing genetic richness at the intraspecific scale may have no effect on energy flow in lentic ecosystems where daphniids are often critical primary consumers. This contrasts with other studies that have documented links between genetic richness at the intraspecific scale in clonal taxa to a variety of ecosystem

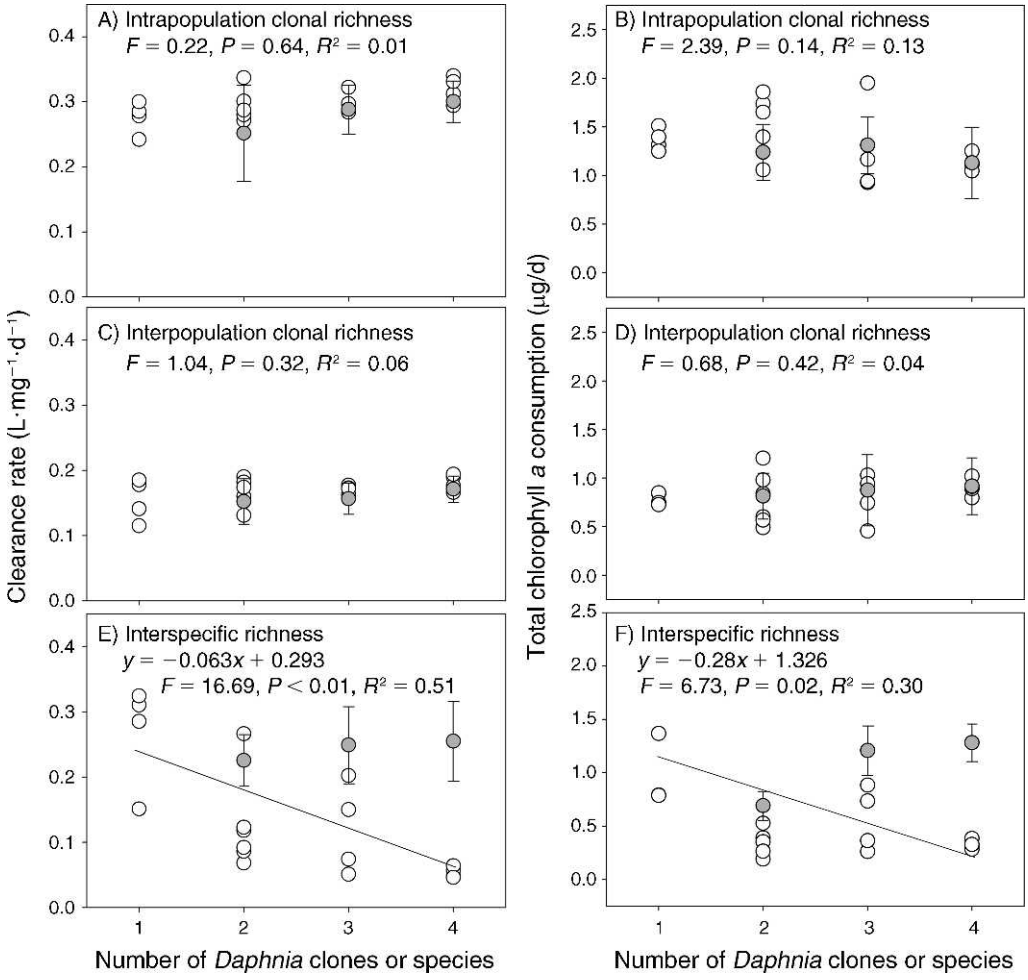

FIG. 1. *Daphnia* assemblage feeding rate and total resource consumption across three levels of increasing genetic richness: (A, B) effects of clonal richness on (A) assemblage clearance rates (CR) and (B) total chlorophyll consumed (TCC) at the intrapopulation level in *D. pulex*; (C, D) effects of clonal richness on (C) assemblage CR and (D) TCC at the interpopulation level in *D. pulicaria*; and (E, F) effects of *Daphnia* species richness on (E) assemblage clearance rates and (F) TCC. Open circles represent the average CR or TCC ($N = 3$ replicates per assemblage combination) for each monoculture assemblage ($N = 4$), and all two- ($N = 6$), three- ($N = 4$), and four- ($N = 4$) clones or taxa assemblage combinations. Shaded circles represent the mean (±95% CI) CR or TCC predicted from a multiplicative risk model (see Eqs. 3 and 4) for all assemblage combinations at a given level of richness.

properties and functions. For example, genetic richness within populations can enhance resistance to parasites, reduce invasibility, increase nutrient cycling, increase resistance to disturbance, and support diversity at consumer trophic levels (reviewed by Hughes et al. 2008). Because our study concentrated only on resource consumption as the ecosystem function, our results do not address other potential regulatory effects of genetic richness in this system. Further research is warranted as some of the ecosystem and community properties presented above also could be linked to genetic richness in *Daphnia*.

The feeding rates of the most species-rich assemblages used in this study (i.e., four species) were similar to rates reported for "whole" *Daphnia* assemblages found in natural lakes (Mourelatos and Lacroix 1990). However, feeding rates of the multispecies assemblages were much reduced compared to monocultures and were ~40% to ~80% lower than expected feeding rates based on predictive models. The difference between the observed and expected feeding rates increased with increasing richness, suggesting that the magnitude of this inhibitory biodiversity effect was greatest in most diverse mixtures. Therefore, contrary to the generally accepted paradigm of the BEF hypothesis (Hooper et al. 2005, Cardinale et al. 2006), our data suggest that lentic ecosystems with high *Daphnia* richness may have lower rates of energy flux from primary producers to consumers than ecosystems with fewer *Daphnia* species.

We used several approaches to explore possible mechanisms driving reduced feeding rate across treatments of increasing *Daphnia* diversity. We found that, in treatments with two or three species, ~50% of the assemblages had feeding rates that were no different

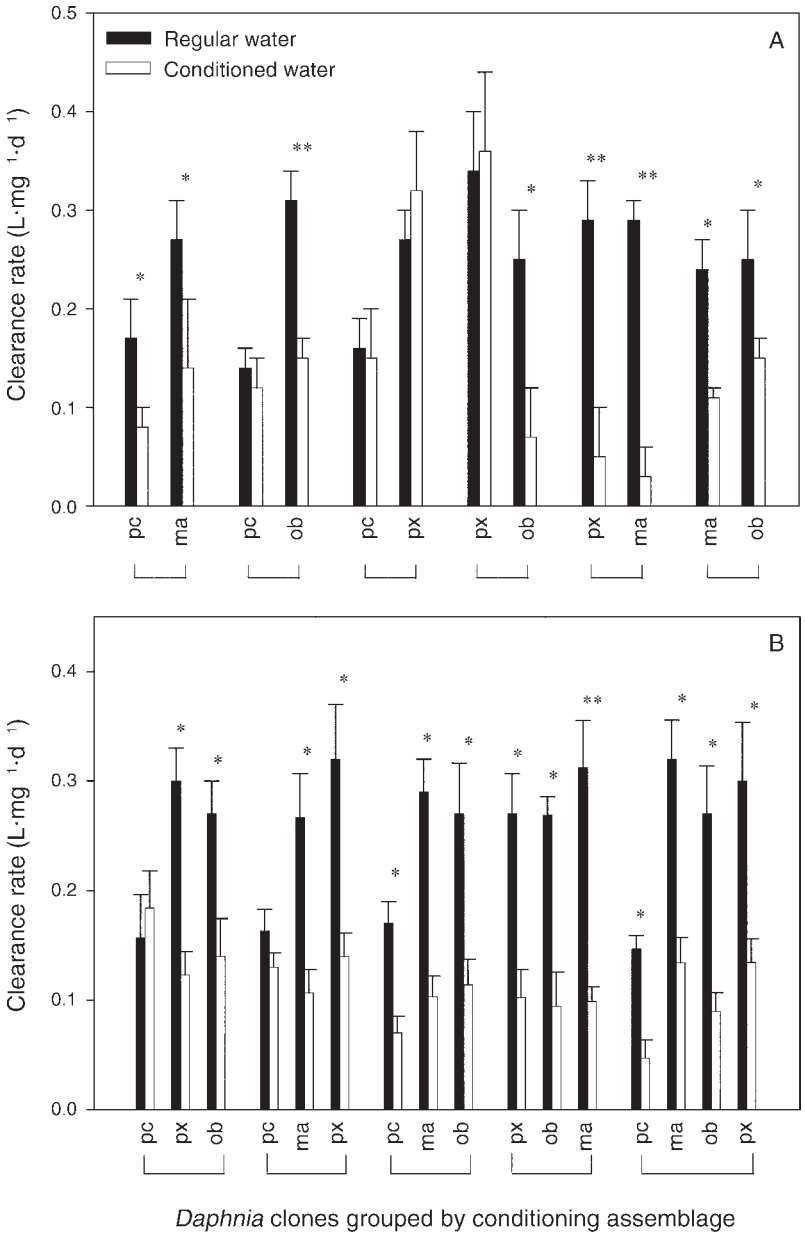

FIG. 2. Mean (+95% CI) monoculture clearance rates (CR) for each *Daphnia* species (where pc indicates *D. pulicaria*, ma indicates *D. magna*, ob indicates *D. obtusa*, and px indicates *D. pulex*) in regular water (solid bars) and in conditioned water (open bars). Conditioned water was created for each two-, three-, and four-species assemblage by adding the different *Daphnia* species to 20-L plastic buckets at a density of 24 individuals/L and allowing them to coexist for 48 h. The species combinations used to make each conditioned water treatment is indicated by brackets on *x*-axes for all (A) two-taxa combinations and (B) three-taxa and four-taxa combinations. Asterisks indicate significant differences between regular and conditioned water for each species.
* $P < 0.05$; ** $P \leq 0.01$.

from the poorest performing species in monoculture. Therefore in these cases, the observed reduction in feeding could simply be explained by the taxa comprising the assemblage. However, in the other ~50% of the two- and three-species combinations and in all of the four-species combinations feeding rates were below that of the poorest performing species in monoculture. This result suggests that in some cases the reduction in assemblage feeding could have been driven by interspecific interactions that inhibit *Daphnia* feeding rates.

We found that the mechanisms responsible for this inhibitory interaction in more species-rich assemblages were likely chemical and mechanical interference between some of the *Daphnia* taxa. For example, in treatments with two species, chemical constituents in the water appeared to be allelopathic, reducing feeding in

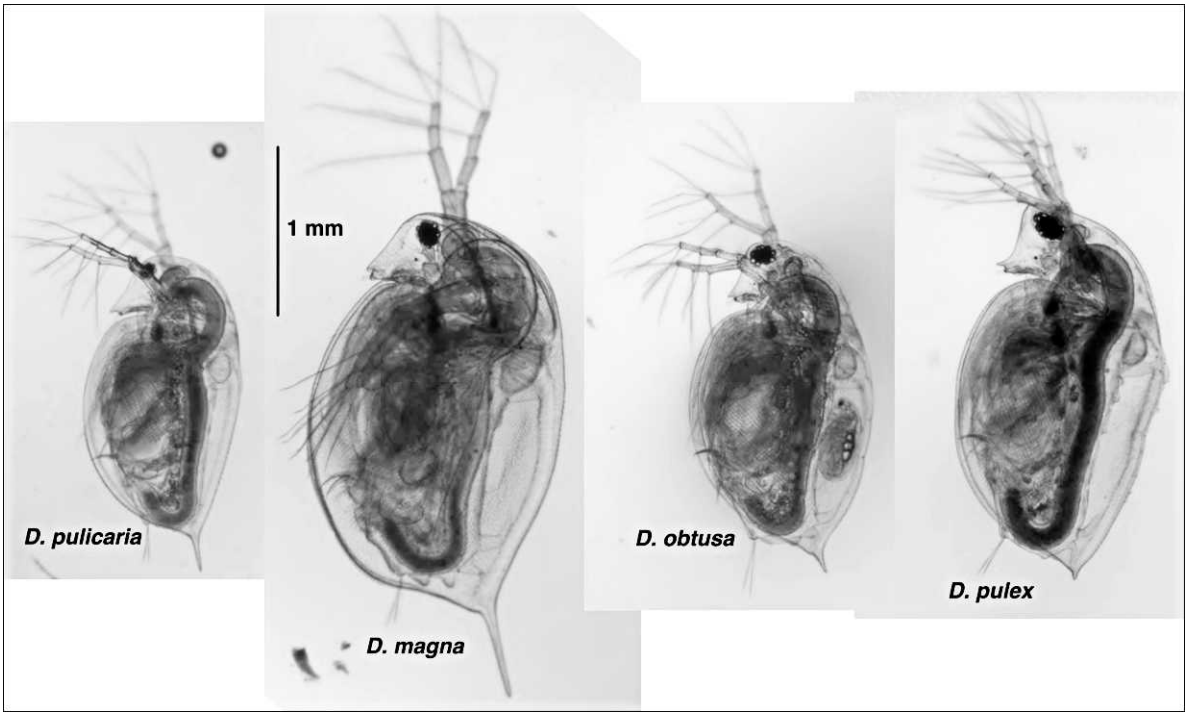

PLATE 1. Four *Daphnia* species used to test effects of increasing genetic richness on resource consumption. Photo credit: K. D. Hambright.

only one of the competing taxa. This, taxon-specific effect resulted in one species driving the observed pattern in these treatments: selection effect. However, as richness of the assemblages increased to four species, inhibitory interactions reduced feeding in all species. The degree of mechanical interference also increased in more species rich assemblages. The simultaneous occurrence of these two interference mechanisms caused an increasing degree of inhibition on *Daphnia* feeding across a gradient of increasing species richness.

Similar inference mechanisms have been linked to dramatic reductions in feeding rates in other zooplankton species such as copepods. For example, Folt and Goldman (1981) reported a 60% reduction in feeding rate of the copepod *Diaptomus tyrelli* in the presence of allelopathic chemicals from potential competitors and predators. In terrestrial systems, competition for homogenous, limiting resources such as water and light often results in allelopathic inhibition of competitors. These mechanisms structure plant assemblages, and inhibit efficient resource use and productivity in some terrestrial ecosystems (Wardle et al. 1998). Our data suggest that interference mechanisms also may structure natural *Daphnia* assemblages over space and time and influence rates of functioning at the ecosystem scale.

Extrapolations of these results could be limited by the temporal and spatial scales inherent to our experimental system. First, the temporal frame was short (24 h) in this study, preventing population-level dynamics that are important for many BEF patterns documented in plants (Duffy et al. 2007). Therefore, our experiment only tested the immediate, short-term interactions among clones and species, and could not address the potential effects of assemblages following multiple generations. It is likely that the interactions we observed in this study would drive dominance of a single species, resulting in greater rates of resource consumption with time. Second, the limited spatial scale of our 2-L experimental bottles could have influenced the intensity of chemical communication, increased likelihood of physical encounters among species, and resulted in less habitat heterogeneity compared to larger experimental systems or lakes. However, *Daphnia* densities used in this study were within the range of natural densities, and, in plants, at least one study showed no effect of experimental plot size on BEF patterns (Roscher et al. 2005). This suggests the spatial scale in this study may have been indicative of natural systems. Finally, interspecific partitioning of heterogeneous resources is one mechanism driving the traditional BEF relationship (Ives et al. 2005). Therefore, the homogonous resource used in this study may have prevented partitioning and exacerbated competition (e.g., Gamfeldt et al. 2005, Snyder et al. 2006). Thus, our study could have overstated the importance of intense interference competition in reducing feeding of natural *Daphnia* assemblages. However, interference mechanisms have been well documented to drive assemblage structure and reduce ecosystem functioning

in plants (Wardle et al. 1998). Species poor *Daphnia* assemblages in nature suggest similar mechanisms could be operating in this consumer system. Therefore, it seems reasonable to assume that the processes observed in this study could be operating in natural assemblages of taxa with high functional overlap or in homogeneous environments.

The BEF paradigm has provided important advances to ecological theory, illustrating that diversity loss will alter function, goods, and services provided by ecosystems (Duffy 2009). In some cases, diversity loss may reduce ecosystem functioning, but our study demonstrates that some assemblages may regulate ecosystem functions more efficiently when composed of fewer taxa. We believe this pattern is more likely to occur when assemblages include functionally similar taxa equipped with mechanisms designed for interference competition (e.g., allelopathy). In this context, competitive processes that drive both spatial and temporal segregation among *Daphnia* (Hu and Tessier 1995, Havel and Lampert 2006) and result in relatively species-poor assemblages in many freshwater ecosystems (Shurin et al. 2007) may actually enhance ecosystem functioning by promoting assemblage dominance by a single clonal species.

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APPENDIX A

Parameter estimates, significance level, and overall model statistics for 10 regression analyses used to calculate clearance rates of separate (clonal) *Daphnia* monocultures (*Ecological Archives* E092-102-A1).

APPENDIX B

Experimental setup showing total *Daphnia* density per bottle, and the individual taxa densities used in the density gradient and end-point approaches (*Ecological Archives* E092-102-A2).

APPENDIX C

Total chlorophyll consumed by each *Daphnia* clone (*Ecological Archives* E092-102-A3).

APPENDIX D

Mean predicted and observed clearance rates and total chlorophyll *a* consumption for each two-, three-, and four-taxa richness combination at the intraspecific level, interpopulation level, and interspecific level (*Ecological Archives* E092-102-A4).

APPENDIX E

The percentage change in clearance rates of each combination assemblage compared to predicted CR based on a multiplicative model, and the average percentage change in CR of all daphniids for each combination in conditioned water compared to their average respective CR in regular (unconditioned) water (*Ecological Archives* E092-102-A5).